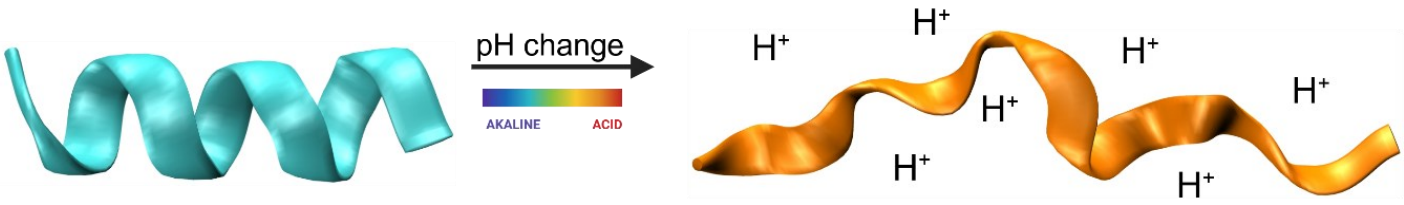
Detection of pH induced structural changes in helical peptides

Silvana S. Zurmühl[1], Anselm H.C. Horn[1,2], Simon Leukel[3], Maike Scherer[4], Kathrin Castiglione[4], Jutta Eichler[3], Heinrich Sticht[1,2]

[1]*Bioinformatics, Institute of Biochemistry, Friedrich-Alexander-Universität Erlangen-Nürnberg*
[2]*Erlangen National High Performance Computing Center (NHR@FAU), Friedrich-Alexander-Universität Erlangen-Nürnberg*

[3]*Department of Chemistry and Pharmacy, Friedrich-Alexander-Universität Erlangen-Nürnberg*

[4]*Department of Chemical and Biological Engineering, Institute of Bioprocess Engineering, Friedrich-Alexander-Universität Erlangen-Nürnberg*



Helices are the most common structural element in proteins, which are often stabilised by interactions between oppositely charged side chains. Consequently, changes in the pH of the solvent, which cause a change in the protonation of these side chains, can lead to destabilisation of the helix, thereby affecting the structure and function of the protein. This is exploited by nature to trigger a variety of physiological processes[1], but can also lead to undesirable processes such as protein aggregation[2]. In addition, pH-dependent helix-coil transitions have the prospect of technical applications including biomaterials and engineered pH switches[3]. For the computational study of the above-mentioned processes, a correct prediction of pH-dependent effects on the structure of α-helices is of utmost importance.

To investigate whether the pH-dependent unfolding of α-helices can be adequately described by MD simulations, we have compiled a set of benchmark peptides known experimentally to undergo a pH-dependent helix-coil transition. We compared the performance of two popular AMBER force fields (ff14SB and ff19SB) with respect to their ability to reproduce the experimentally observed difference in helical content (HC). Each simulation was carried out over a time span of 1 µs and was simulated in triplicate in order to increase the reproducibility and structural convergence. Our simulations show that both ff14SB and ff19SB are able to detect pH-dependent structural changes for the benchmark peptides. However, on a quantitative level, there are distinct differences between the two force fields with respect to the change in helical content or the relative strength of polar intramolecular interactions. Finally, we investigated the performance of ff14SB and ff19SB for two peptides (O13 and O16) that have been optimised for helix stability, but the pH dependence of their stability is unknown.[4] Both force fields model a decrease in HC at acidic pH for O13. We were able to confirm these observations with circular dichroism (CD) spectroscopy measurements of in-house synthesised peptides. All simulations of O16 show a consistently high HC under different pH conditions, suggesting that O16 is stable at basic and acidic pH. Our experimental measurements corroborate these findings. Thus, our results indicate that both force fields, ff14SB and ff19SB, can be used to identify pH-dependent switches in peptides.

[1] L. Luzia, D. Lao-Martil, P. Savakis, J. van Heerden, N. van Riel, B. Teusink, *The FEBS Journal* **2022**, *289*, 6021–6037.
[2] P. Picotti, G. De Franceschi, E. Frare, B. Spolaore, M. Zambonin, F. Chiti, P. P. de Laureto, A. Fontana, *Journal of Molecular Biology* **2007**, *367*, 1237–1245.
[3] S. Pavan, F. Berti, *Anal Bioanal Chem* **2012**, *402*, 3055–3070.
[4] M. Petukhov, Y. Tatsu, K. Tamaki, S. Murase, H. Uekawa, S. Yoshikawa, L. Serrano, N. Yumoto, *Journal of Peptide Science* **2009**, *15*, 359–365.